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REMARKS

The present response is intended to be fully responsive to all points of objection and/or rejection raised by the Examiner and is believed to place the application in condition for allowance. Favorable reconsideration and allowance of the application is respectfully requested.

Status of Claims

Claims 21-25 are pending in the application. Claims 21-25 have been rejected. Claims 21 and 22 have been amended. Claims 26-34 have been added.

Claims 23-24 have been canceled without prejudice or disclaimer. In making this cancellation without prejudice, Applicants reserve all rights in these claims to file divisional and/or continuation patent applications.

CLAIM REJECTIONS

35 U.S.C. § 112, Second Paragraph Rejections

In the Office Action, the Examiner rejected claims 21-25 under 35 U.S.C. § 112, second paragraph, as being indefinite for allegedly failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner alleged that the recitation of "substantially free" in claim 21 renders the claim indefinite.

In response, in order to expedite prosecution, amended claim 21 is directed to composition comprising a culture medium, the culture medium being free of animal products and comprising a yeast extract, the composition further comprising *Clostridium difficile*. New claim 27 is directed to a composition comprising a culture medium, the culture medium being free of animal products and comprising a soy product, the composition further comprising *Clostridium difficile*. New claims 28 and 29 recite that the soy product is a hydrolyzed soy product or a soy peptone, respectively. New claim 31 is directed to a composition comprising a culture medium, the culture medium being free of animal products and comprising sodium thioglycolate, the composition further comprising *Clostridium difficile*. Support for amended claim 21 and new claim 31 is found in the subject specification as filed on page 3, lines 1-2,

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and in the first full paragraph on page 7. Support for new claims 27-29 is found in the subject specification in the paragraph beginning on page 3.

Applicants therefore respectfully request that the Examiner withdraw the rejection.

Further, the Examiner rejected claim 22 under 35 U.S.C. § 112, second paragraph, alleging that recitation of the term "derived from" renders the claim indefinite.

In response, in order to expedite prosecution but without agreeing to the rejection, amended claim 22 is directed to composition comprising a culture medium, the culture medium being free of animal products and comprising a yeast extract and a compound from a vegetable, the composition further comprising *Clostridium difficile*.

Applicants therefore respectfully request that the Examiner withdraw the rejection.

Further, the Examiner rejected claim 24 under 35 U.S.C. § 112, second paragraph, alleging that hydrolyzed soy is not a "compound" but rather a complex mix of compounds.

Claim 24 has been canceled for reasons unrelated to the rejection of claim 24 under 35 U.S.C. § 112, second paragraph, rendering the rejection moot.

Applicants therefore respectfully request that the Examiner withdraw the rejection.

35 U.S.C. § 102 Rejections

Further, the Examiner rejected claims 21, 22, and 25 under 35 U.S.C. § 102(b), as being allegedly anticipated by either of the Castell references. The Examiner alleged that the Castell references disclose a culture medium substantially free of animal products and comprising *C. difficile*. The Examiner admitted, however, that the subject matter of claims 23 and 24 is not disclosed in either of the Castell references.

In response, Applicants agree that claims 23 and 24 are not disclosed in either of the Castell references and respectfully traverse the rejection. Amended claim 21 is directed to a composition comprising a culture medium, the culture medium being substantially free of animal products and comprising a yeast extract, the composition further comprising *Clostridium difficile*. New claim 27 is directed to a composition comprising a culture medium, the culture medium being substantially free of animal products and comprising a

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soy product, the composition further comprising *Clostridium difficile*. New claim 31 is directed to a composition comprising a culture medium, the culture medium being substantially free of animal products and comprising sodium thioglycolate, the composition further comprising *Clostridium difficile*. None of the limitations "yeast extract," "soy product," "sodium thioglycolate" (claims 21, 27, and 31) are disclosed or suggested in either of the Castell references.

Applicants therefore respectfully request that the Examiner withdraw the rejection.

Further, the Examiner rejected claims 21, 22, and 25 under 35 U.S.C. § 102(b), as being allegedly anticipated by Saif et al. The Examiner alleged that Saif discloses on page 135-136 a culture medium substantially free of animal products and comprising *C. difficile*. The Examiner admitted, however, that the subject matter of claims 23 and 24 is not disclosed in Saif.

In response, Applicants agree that claims 23 and 24 are not disclosed in Saif and respectfully disagree with the rejection. Contrary to Examiner's allegations, Saif does not disclose on pages 135-136 a culture medium substantially free of animal products and comprising *C. difficile*. At the most, Saif discloses isolation of *C. difficile* from water samples and other natural specimens. Saif does not disclose growth of *C. difficile* in any of the water samples or other natural specimens allegedly disclosed on pages 135-136. Thus, Saif does not disclose on pages 135-136 a culture medium substantially free of animal products and comprising *C. difficile*.

Further, the culture medium utilized by Saif to grow *C. difficile*, Brazier's CCEY agar, is prepared with lysed horse blood and egg yolk emulsion X073, as described in the attached leaflet. Thus, contrary to the Examiner's allegations, the culture medium utilized by Saif is not "substantially free of animal products."

Applicants therefore respectfully request that the Examiner withdraw the rejection.

35 U.S.C. § 103 Rejections

In the Office Action, the Examiner rejected claims 23 and 24 under 35 U.S.C. § 103(a), as being unpatentable over either of the Castell references in combination with the

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Ingram reference. The Examiner alleged that, in view of Ingram, it would have been obvious to one skilled in the art to modify the culture media of either of the Castell references by adding a soy hydrolysate as an additional source of nutrients.

Applicants respectfully disagree. Ingram is directed to production of ethanol by ethanologenic bacteria, as clearly indicated therein:

“Applicants have discovered a method for the production of ethanol comprising (a) contacting a nutrient medium selected from the group consisting of a pasteurized, hydrolyzed soy product and a pasteurized, autolyzed yeast product, ethanologenic bacteria and a fermentable sugar, thereby obtaining a mixture and (b) incubating said mixture under conditions suitable for the production of ethanol” (column 2, lines 33-40; emphasis added)

Further, the abstract and independent claims 1 and 9 of Ingram also recite “Ethanologenic bacteria.” Thus, Ingram is totally unconnected with the subject claims, which are directed to a C. difficile culture medium. Accordingly, Ingram should not be considered a prior art reference for the subject invention. Further, a person skilled in the art would have no motivation to combine Ingram with either of the Castell references, as they are directed to completely different and non-overlapping subject matter.

Further, the present invention disclosed unexpected results, namely, far superior Toxin A production after 3 days, compared with vegetable peptone and other non-animal sources, as disclosed in the subject specification in Table 6 (page 14).

Further, the culture medium recited in the subject claims fulfilled a long-felt need in the art; namely, provision of a culture media for culturing C. difficile that is free of animal products, and thus does not engender a fear of undesirable contaminants that may be present in animal products, as described in the subject specification (page 3, first paragraph).

Thus, the subject matter of the subject claims is clearly non-obvious in view either of the Castell references, either alone or in combination with Ingram.

Applicants therefore respectfully request that the Examiner withdraw the rejection.

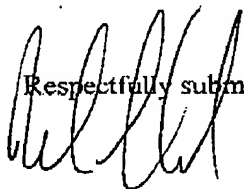
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In view of the foregoing amendments and remarks, the pending claims are deemed to be allowable. Their favorable reconsideration and allowance is respectfully requested.

Should the Examiner have any question or comment as to the form, content or entry of this Amendment, the Examiner is requested to contact the undersigned at the telephone number below. Similarly, if there are any further issues yet to be resolved to advance the prosecution of this application to issue, the Examiner is requested to telephone the undersigned counsel.

Please charge any fees associated with this paper to deposit account No. 50-3355.

Respectfully submitted,



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Brazier's CCEY Agar

LAB 160

Description

Brazier's CCEY agar is the formulation currently used by the Anaerobe Reference Unit for the isolation of *C. difficile*, resulting from work initiated by Ken Phillips and Paul Tzevetz, and completed by Jon Brazier.

Based upon the market leading anaerobe medium, Fastidious Anaerobe Agar, it incorporates additional ingredients to improve the isolation and differentiation of *C. difficile* from clinical specimens.

Cholic acid is present to promote spore germination following alcohol shock treatment, and p-hydroxyphenylacetic acid to enhance the production of p-cresol, a distinctive metabolite of *C. difficile*.

Selectivity is achieved by addition of supplement X093 (cefotaxime cycloserine) and egg yolk emulsion X073 is added to help differentiate *C. difficile* from lecithinase positive clostridia. Finally the addition of lysed horse blood optimises the recognition of colony fluorescence when cultures are examined using UV light.

| Formula | g/litre |
|----------------------------|---------|
| Peptone Mix | 23.0 |
| Sodium chloride | 5.0 |
| Soluble Starch | 1.0 |
| Agar | 12.0 |
| Sodium bicarbonate | 0.4 |
| Glucose | 1.0 |
| Sodium pyruvate | 1.0 |
| Cysteine HCl | 0.5 |
| Haemin | 0.01 |
| Vitamin K | 0.001 |
| L-arginine | 1.0 |
| Soluble pyrophosphate | 0.25 |
| Sodium succinate | 0.5 |
| Cholic acid | 1.0 |
| p-Hydroxyphenylacetic acid | 1.0 |

Method for reconstitution

Weigh 48 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix, and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and aseptically add the following: 2 vials of X093, 40ml of Egg Yolk Emulsion X073 and 10ml lysed horse blood. Mix well and pour into Petri dishes.

Appearance: Tan opaque gel.

pH: 7.0 ± 0.2

Minimum O.C. organisms: *C. difficile*
E. coli (inhibition) NCIMB 50034

Storage of prepared medium: Plates – up to 7 days at 2-8°C in the dark.

Inoculation: Surface streak untreated or alcohol shocked specimens for single colonies.

Incubation: 37°C for 24-48hrs under anaerobic conditions

Characteristics of *C. difficile*: Gray opaque flat colonies, raised elevation, 2-3mm diameter, generally circular but tending to elongate in the direction of spreading, ground glass appearance and a rough, fimbriate edge. Lecithinase negative. Incubation longer than 48hrs may result in a lighter gray or white centre to the colony. Phenolic odour due to the production of p-cresol. Colonies fluoresce yellowgreen under UV light. Confirm by latex agglutination.

References

Brazier J.S. (1993) Role of the Laboratory in Investigations of Clostridium difficile Diarrhoea. Clinical Infectious Diseases 16 (4) 228-33.

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